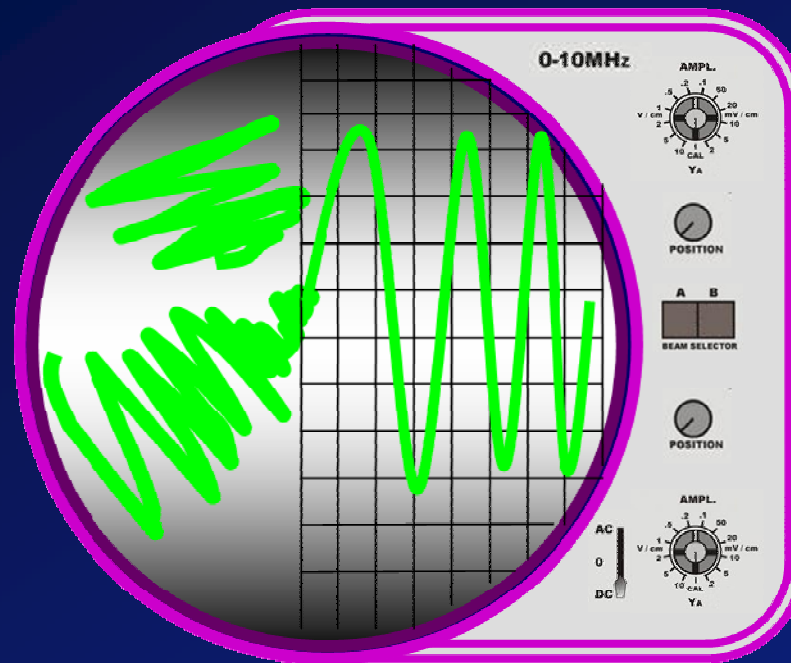


iGEM Jamboree
MIT – Nov 4th 2006





Engineering a Molecular Predation Oscillator



iGEM 2006 @ Imperial



Biomedical
Engineers



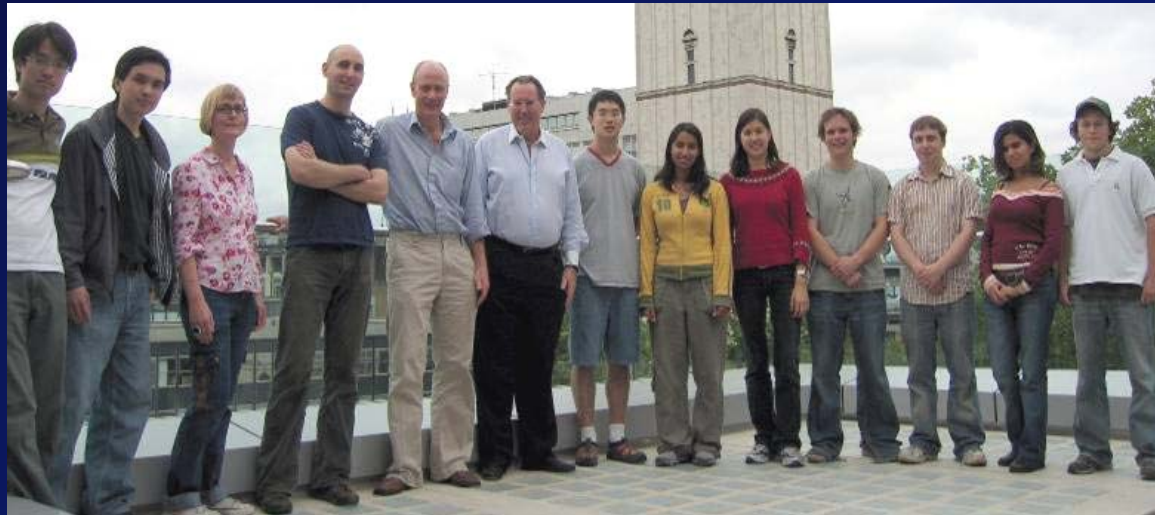
Electrical
Engineer



Biochemist



Biologists



Biomedical
Engineers

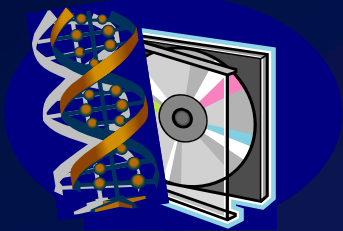
Biochemists



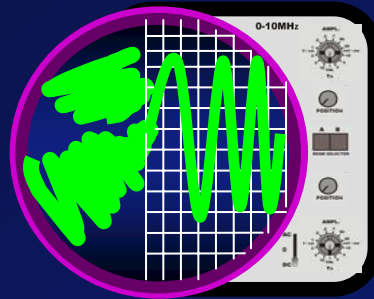
Dr Mann



Project Ideas



Bio-Memory



Oscillator



Bio-Clock

- Feasibility
- Originality of Design
- BioBrick Availability
- Future Impact



What is an Oscillator?

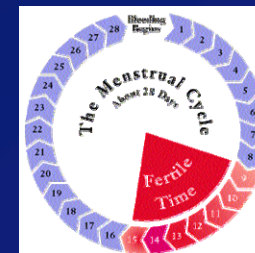
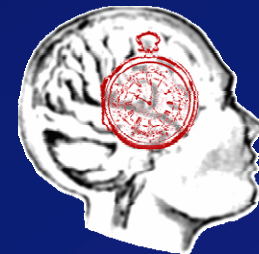
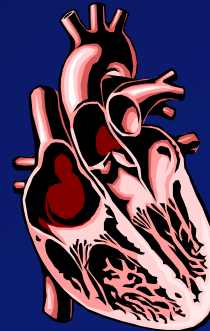
Our Definition

Device producing a periodic variation in time of a measurable quantity, e.g. amplitude.

Engineering



Biology



The Engineering Approach





The Main Challenges

Main challenges of past oscillators:

- Unstable
- Noisy
- Inflexible

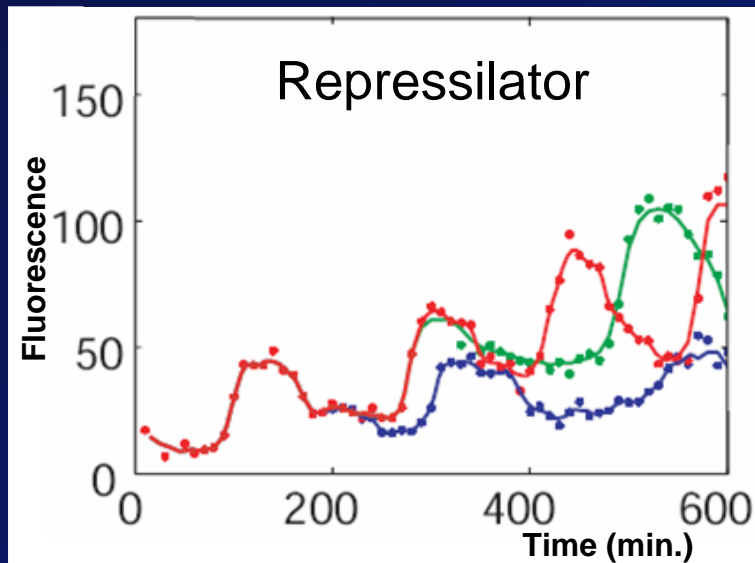


Figure Reference : Michael B. Elowitz & Stanislas Leibler *Nature* 2000

Requirement for a typical engineering oscillator: Our Specifications:

- Sustained Oscillations
- **Stability**: > 10 periods
- **SNR**: Signal to Noise Ratio
- **Flexibility**: Controllable Amplitude and Frequency
- Standardized Device for **Modular Design**
- Easy Connectivity
- **Easy Connectivity**



Our Initial Design Ideas

Based on

- Large populations of molecules to reduce influence of noise
- Oscillations due to population dynamics
- A well characterized model

Molecular Predator - Prey

The Lotka-Volterra Model

$$\frac{dY}{dt}$$

=

Prey Growth



—

Prey Killing
by Predator



$$\frac{dV}{dt}$$

=

Predator Growth

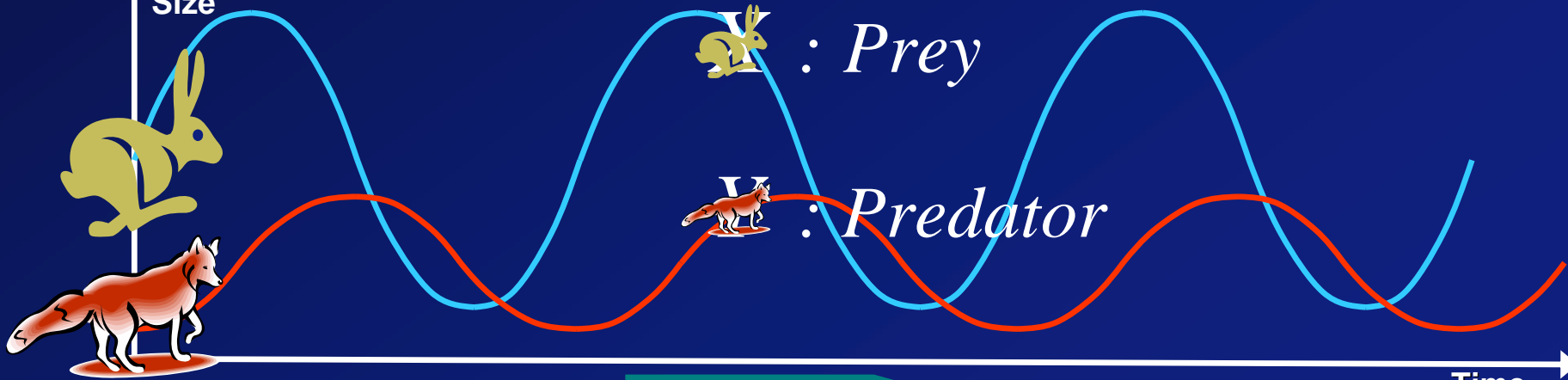


—

Predator Death



Population
Size



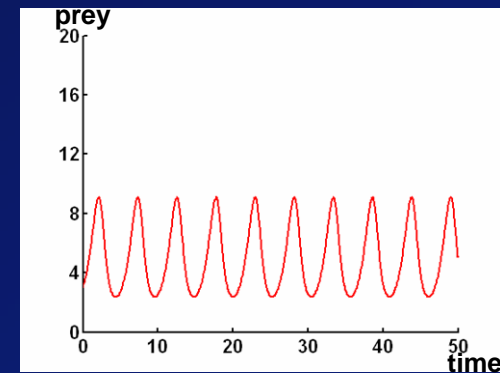
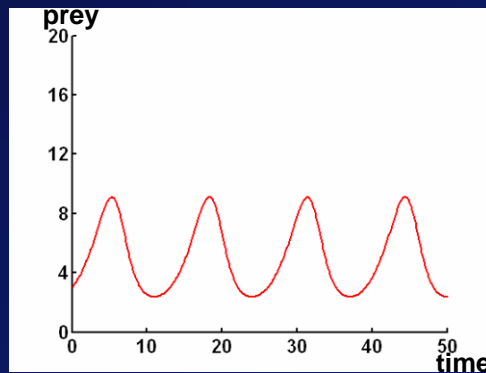
Typical LV Simulations

**Graph of
Prey vs. Time**

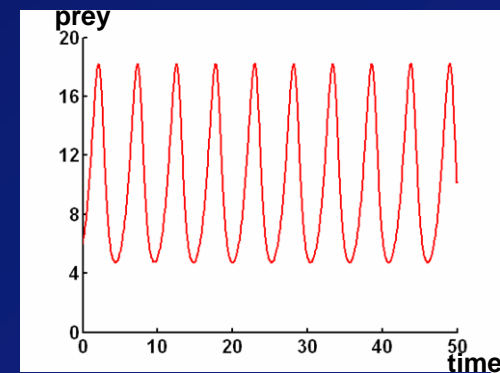
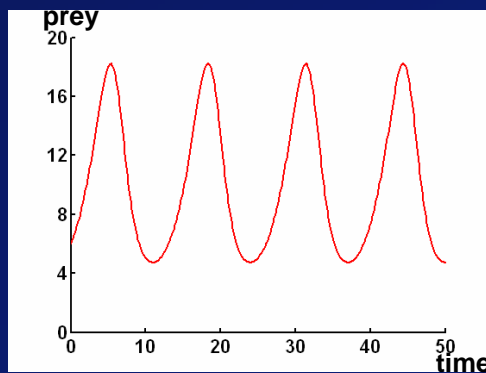
Low Frequency

High Frequency

**Small
Amplitude**



**Large
Amplitude**





Required Biochemical Properties

$$\frac{d \text{ (Rabbit) } A}{dt}$$

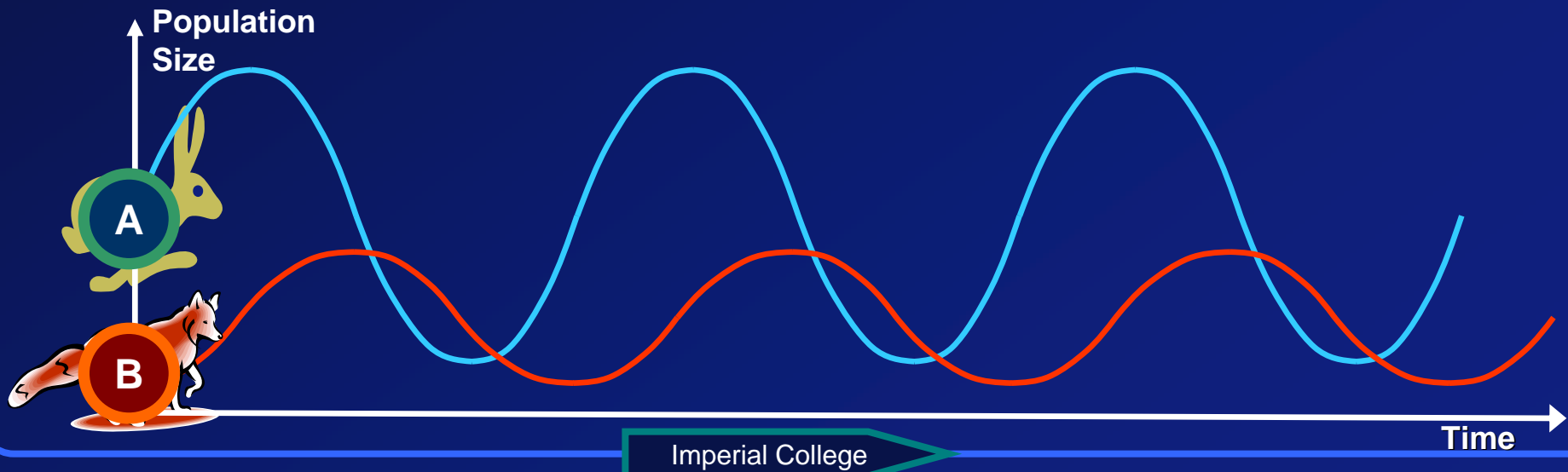
Self promoted
expression of A

Degradation
of A by B

$$\frac{d \text{ (Fox) } B}{dt}$$

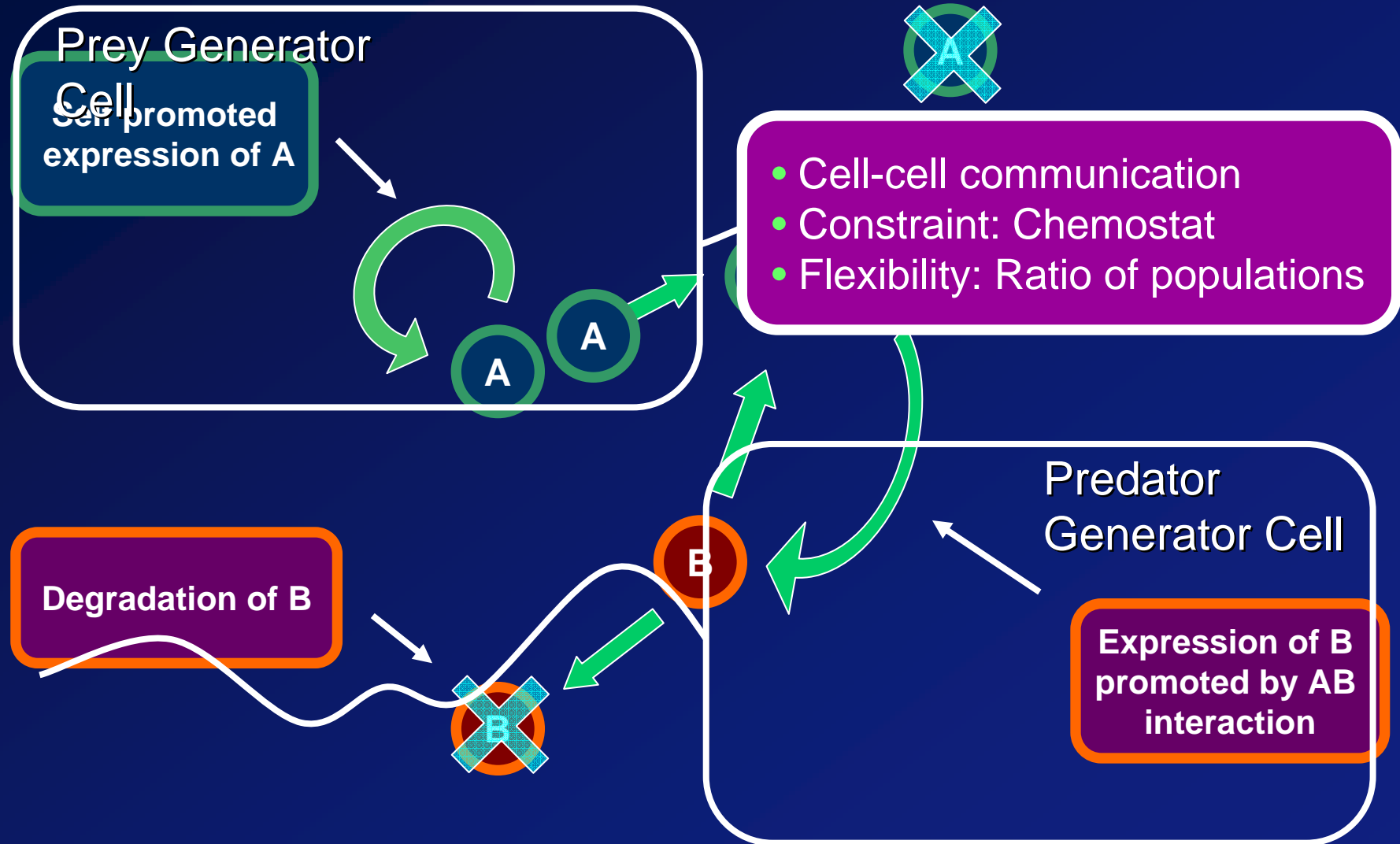
Expression of B
promoted by
AB interaction

Degradation
of B



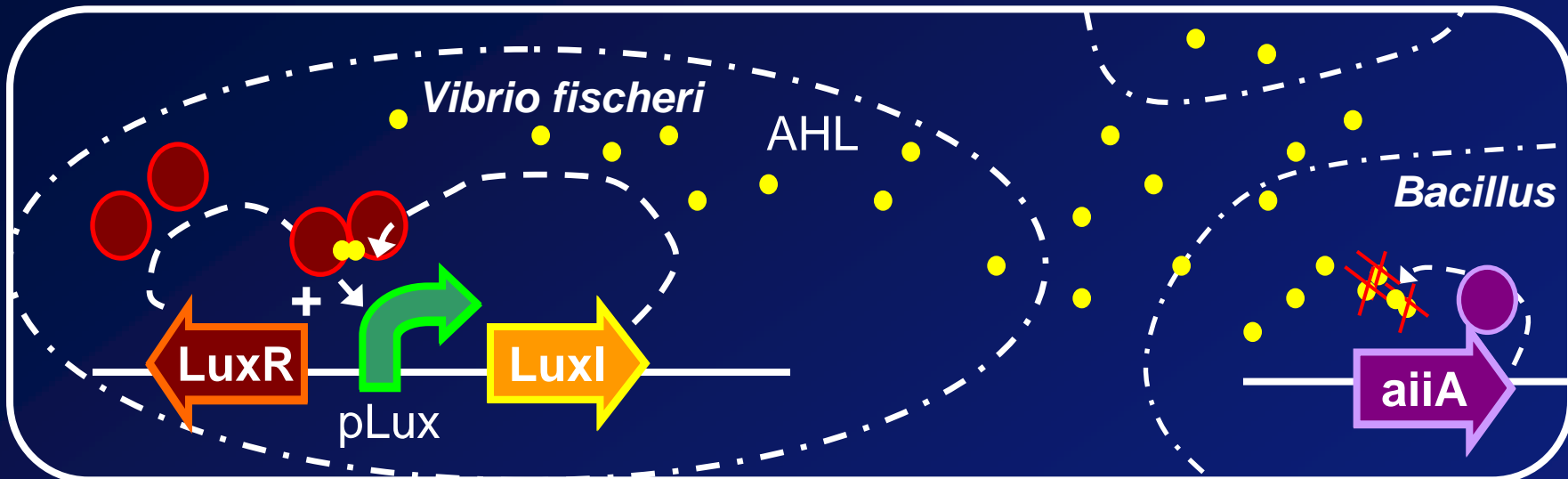


Molecular System





Quorum sensing/quenching



BioBricks available

BBa_C0062	Forms a complex with AHL to activate pLux	BBa_C0061	Makes AHL
pLux BBa_R0062	pLux Promoter	BBa_C0160	Degrades AHL
<p>BBa_F2620</p>		AHL->Pops Receiver	



Designing the Prey Generator

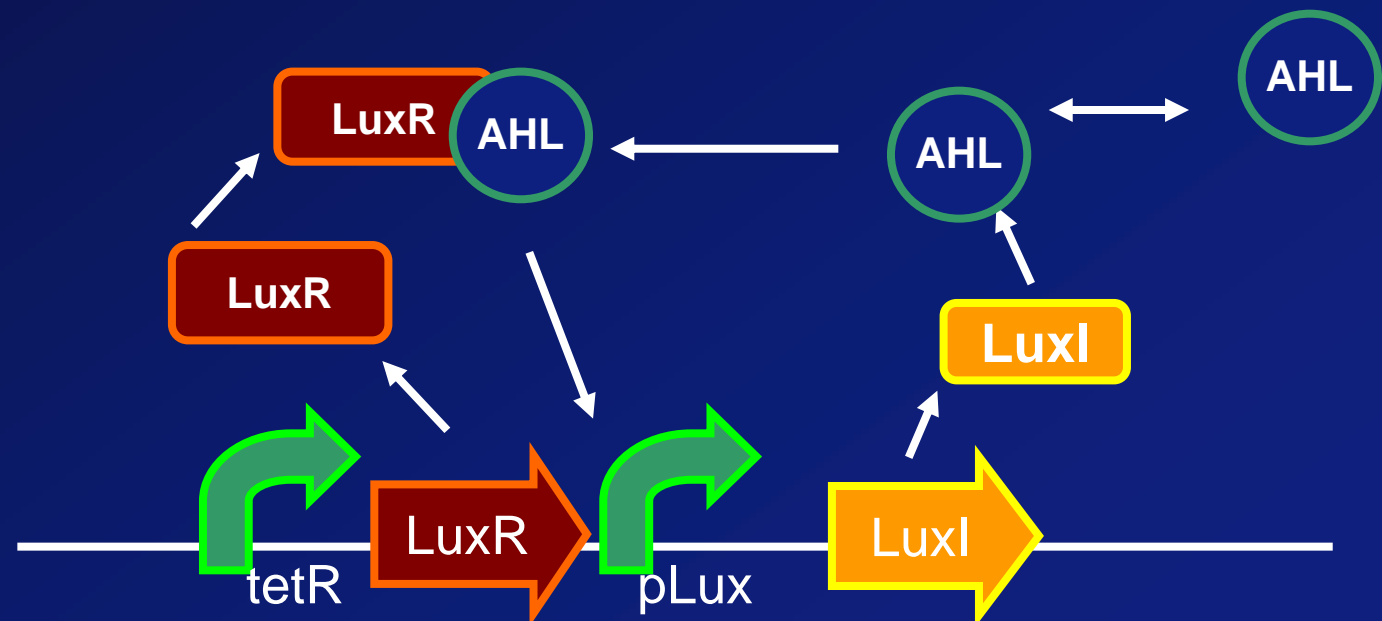
Required
Dynamic

Self promoted
expression of A ✓

Useful
BioBricks



Final
Construct





Designing the Predator Generator

**Required
Dynamic**

Expression of B
promoted by
AB interaction ✓

Degradation
of A by B ✓

Degradation
of B ✓

**Useful
BioBricks**

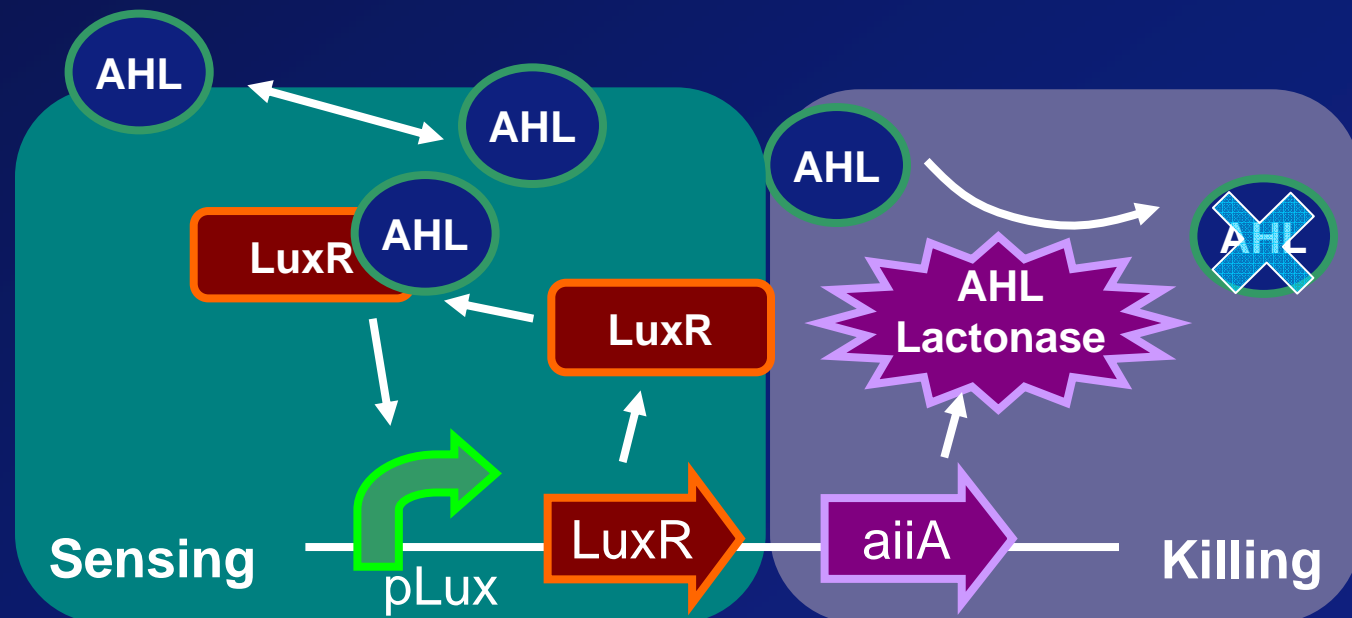

pLux
R0062


LuxR
C0062


aiiA
C0060

Natural
degradation

**Final
Construct**



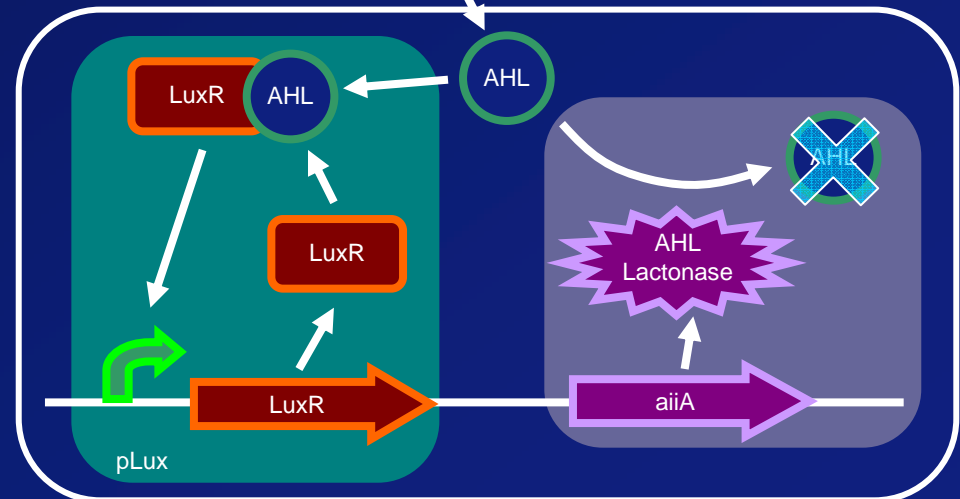
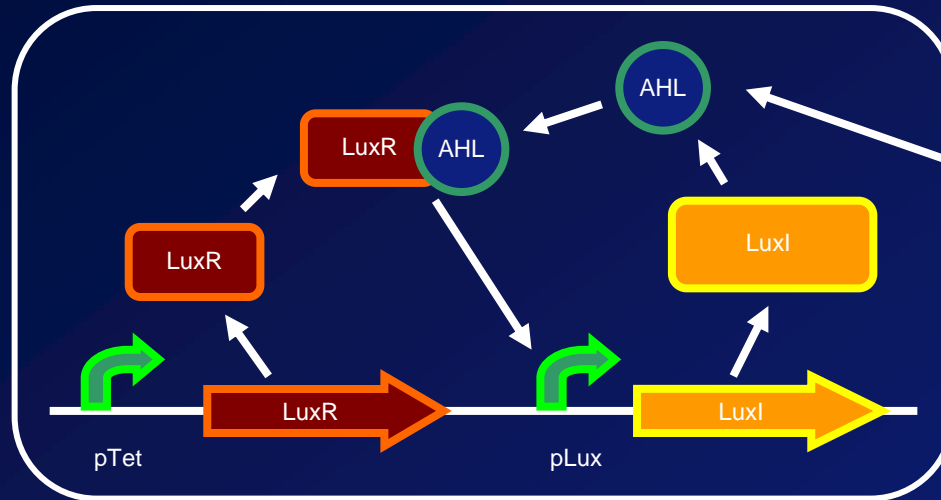


System Overview

Prey Generator Cell

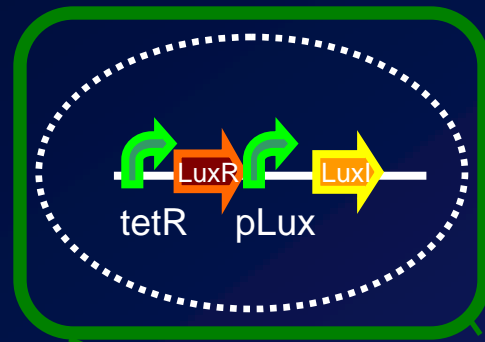
Pool of AHL will oscillate

Predator Generator Cell

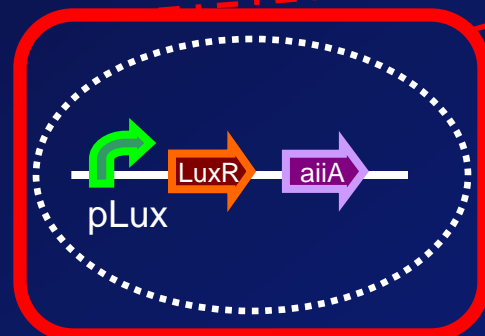




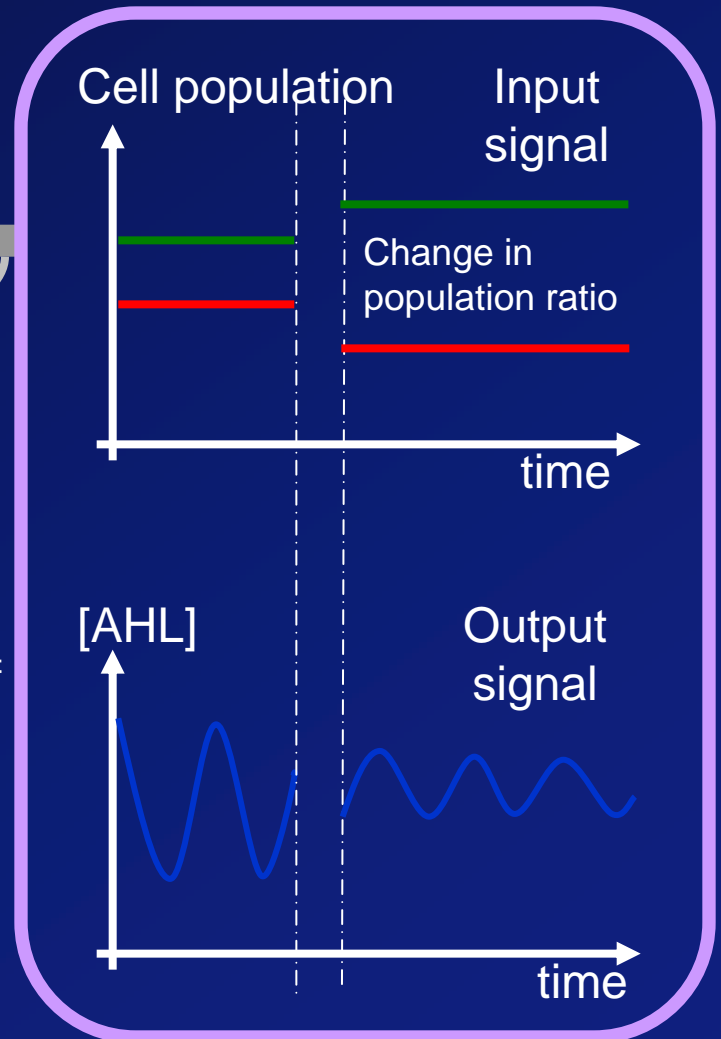
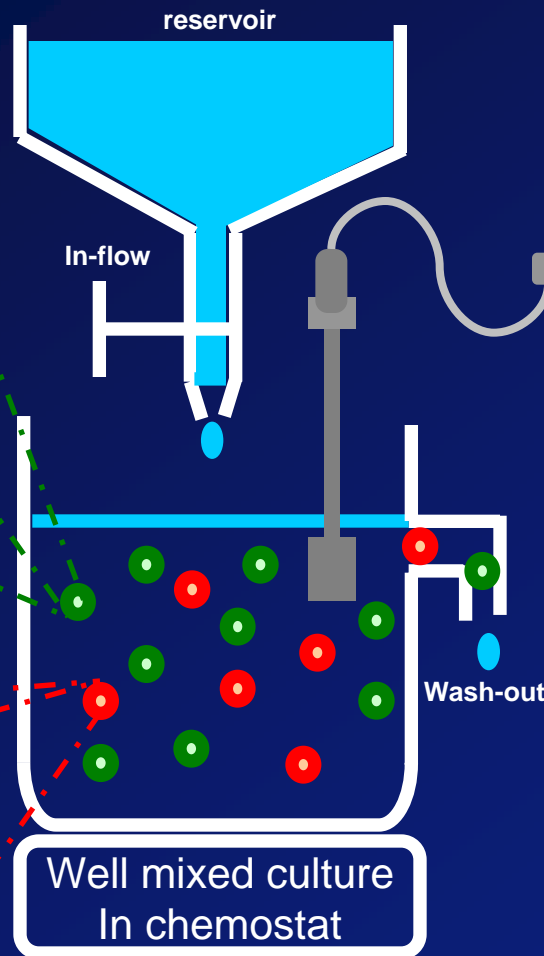
Full System set-up



Prey molecule generator

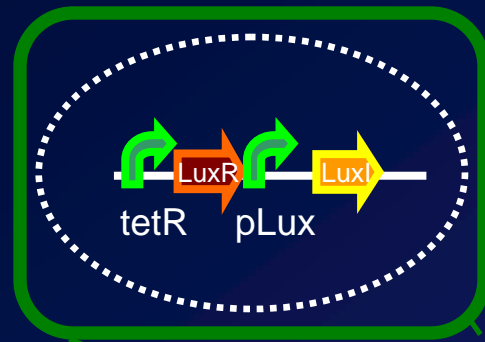


Predator molecule generator

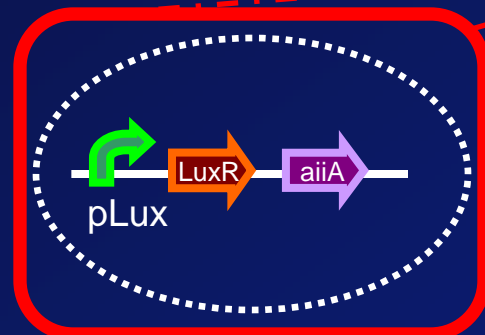
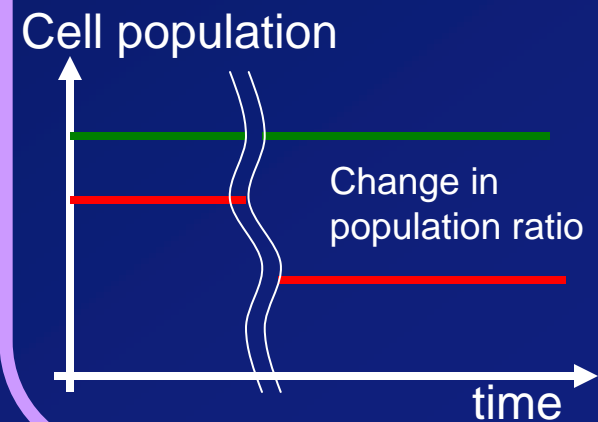
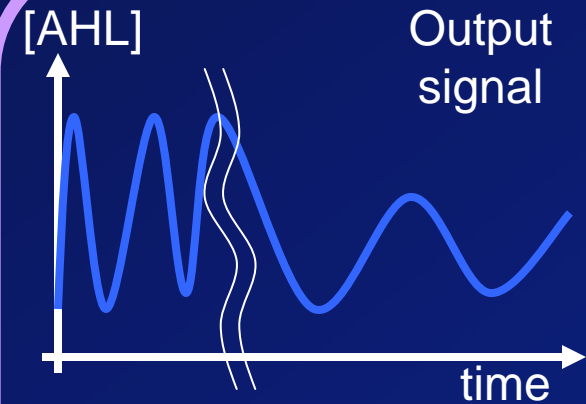
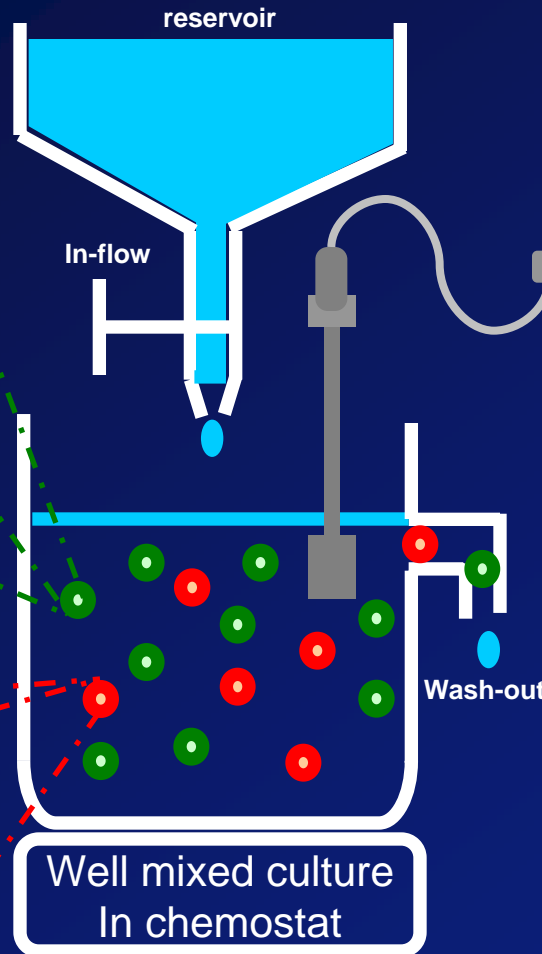




Full System set-up



Prey molecule generator

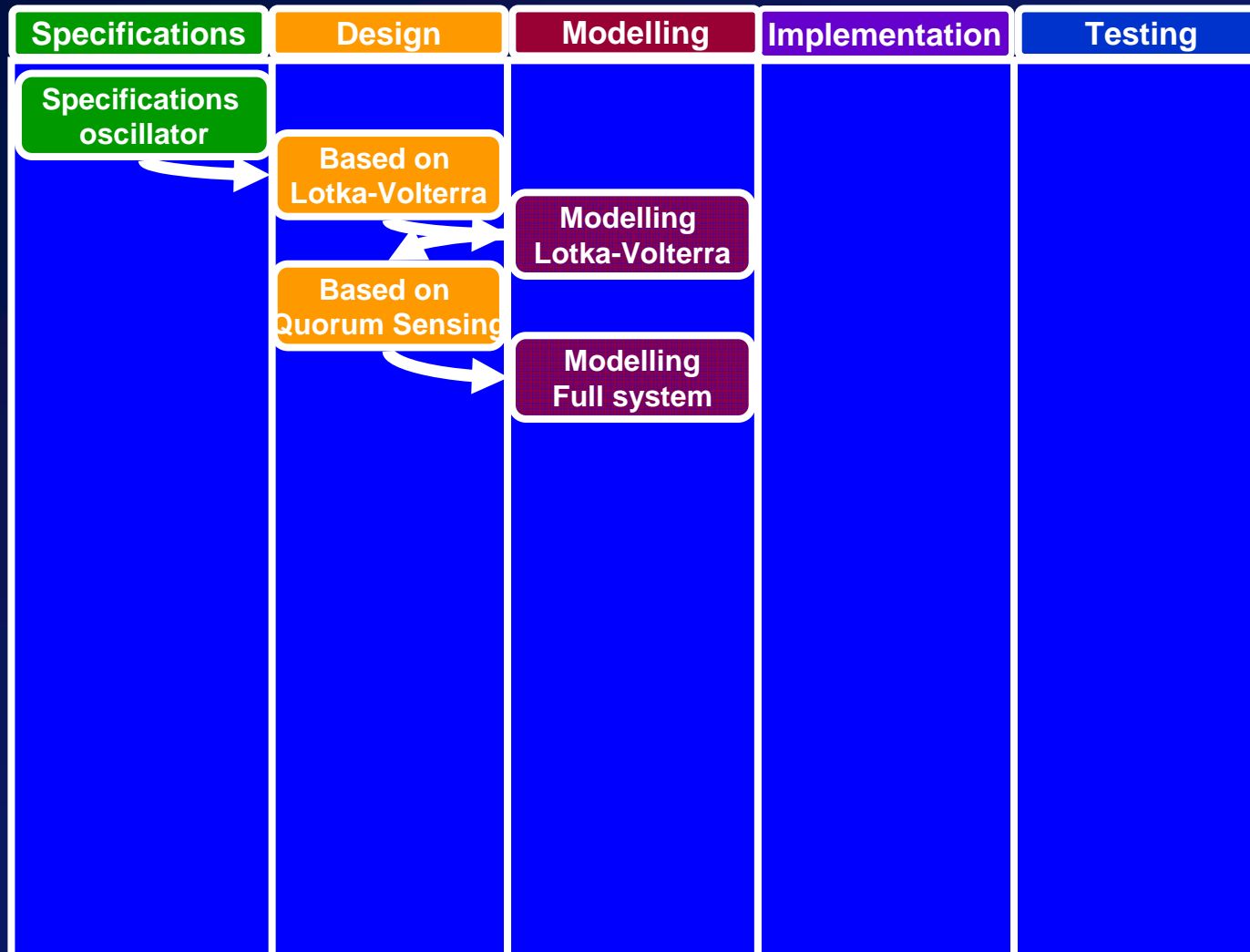


Predator molecule generator



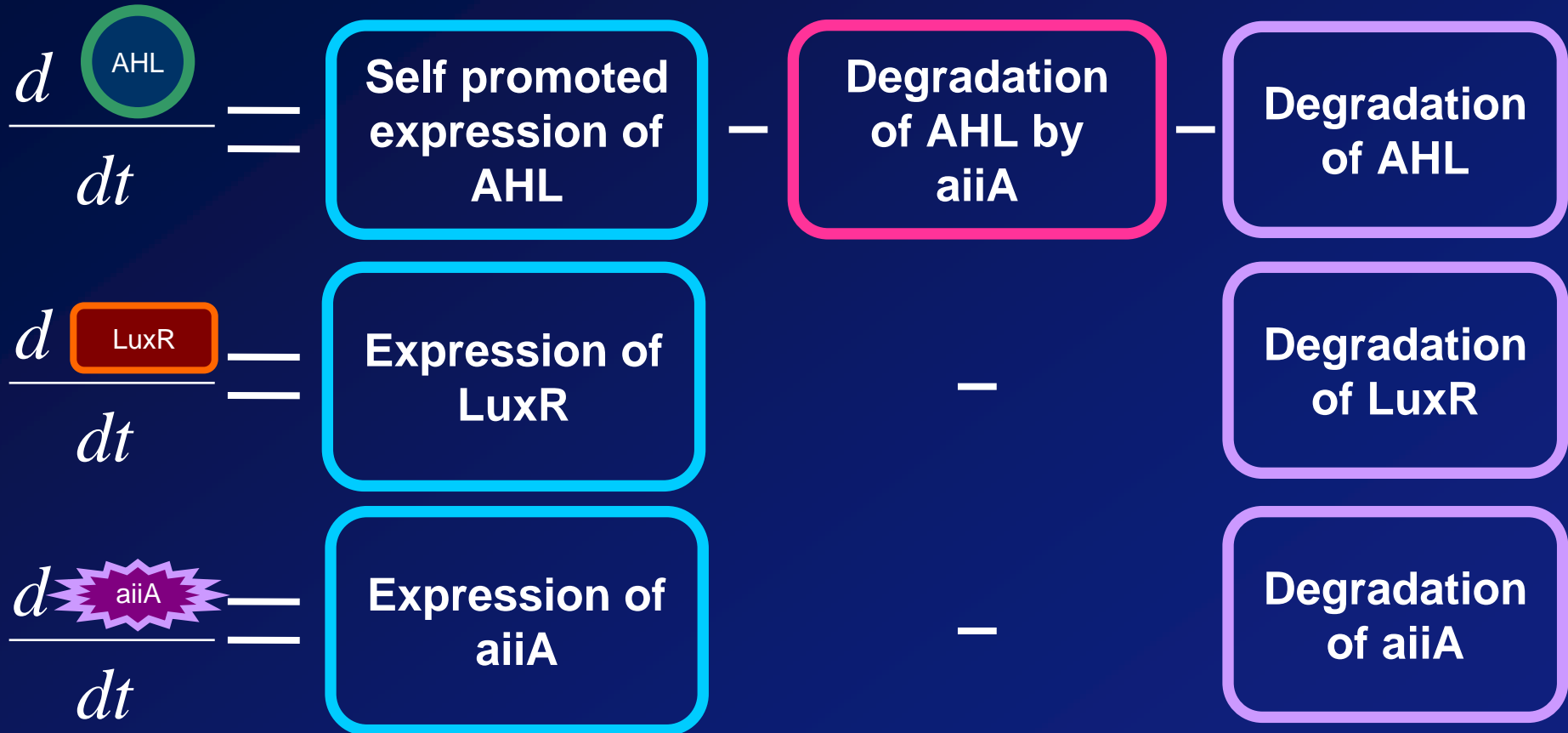
Path to Our Goal

Start!
→



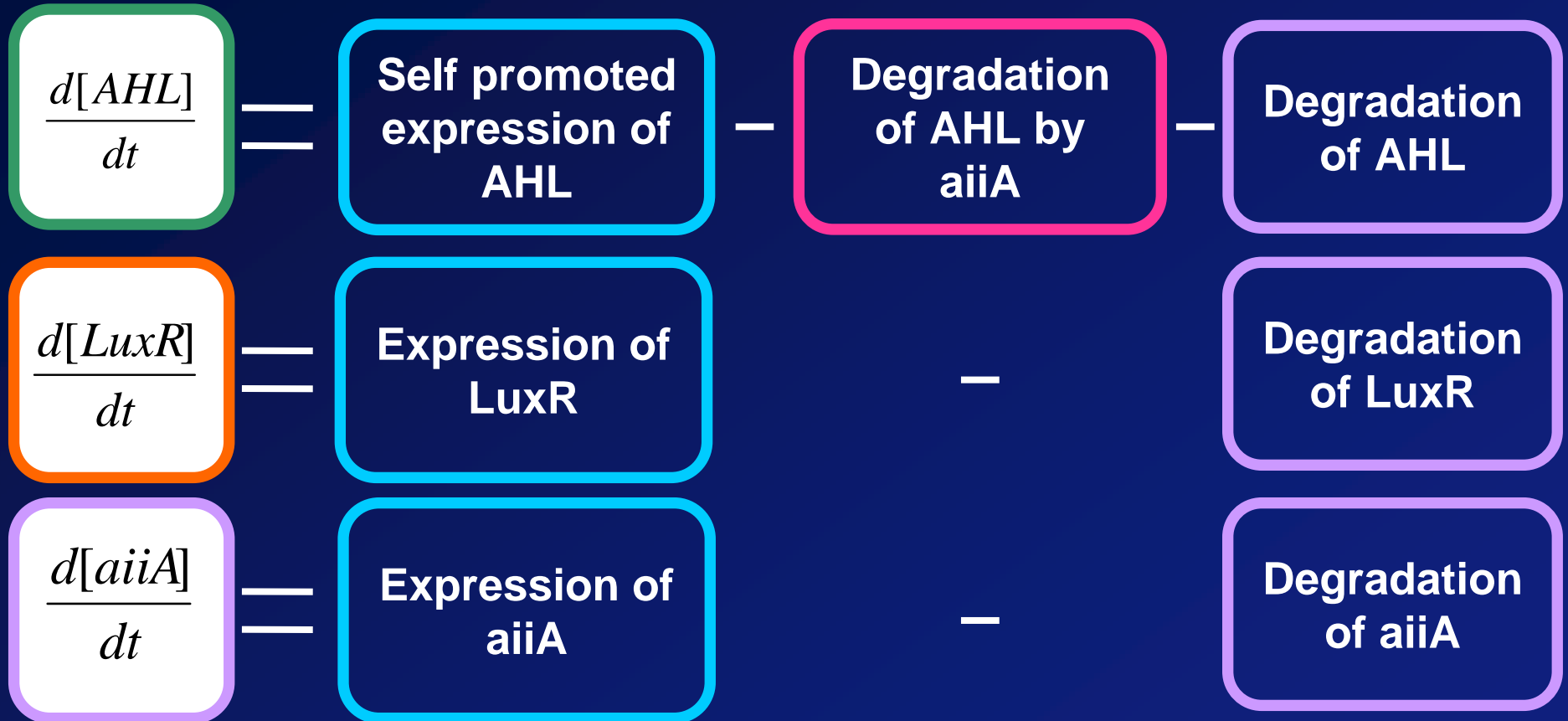


Modelling the Full System





Modelling the Full System





Modelling the Full System

Gene Expression

$$\frac{d[AHL]}{dt}$$

=

$$\frac{a[AHL]}{a_0 + [AHL]}$$

—

Degradation
of AHL by
aIIA

—

Degradation
of AHL

$$\frac{d[LuxR]}{dt}$$

=

$$\frac{c[AHL][LuxR]}{c_0 + [AHL][LuxR]}$$

—

Degradation
of LuxR

$$\frac{d[aIIA]}{dt}$$

=

$$\frac{c[AHL][LuxR]}{c_0 + [AHL][LuxR]}$$

—

Degradation
of aIIA



Modelling the Full System

Gene Expression

Enzymatic Reaction

$$\frac{d[AHL]}{dt}$$

=

$$\frac{a[AHL]}{a_0 + [AHL]}$$

—

$$\frac{b[aiiA][AHL]}{b_0 + [AHL]}$$

—

Degradation
of AHL

$$\frac{d[LuxR]}{dt}$$

=

$$\frac{c[AHL][LuxR]}{c_0 + [AHL][LuxR]}$$

—

Degradation
of LuxR

$$\frac{d[aiiA]}{dt}$$

=

$$\frac{c[AHL][LuxR]}{c_0 + [AHL][LuxR]}$$

—

Degradation
of aiiA



Modelling the Full System

Gene Expression

Enzymatic Reaction

Degradation

$$\frac{d[AHL]}{dt}$$

=

$$\frac{a[AHL]}{a_0 + [AHL]}$$

-

$$\frac{b[aiiA][AHL]}{b_0 + [AHL]}$$

-

$$e[AHL]$$

$$\frac{d[LuxR]}{dt}$$

=

$$\frac{c[AHL][LuxR]}{c_0 + [AHL][LuxR]}$$

-

$$d_1[LuxR]$$

$$\frac{d[aiiA]}{dt}$$

=

$$\frac{c[AHL][LuxR]}{c_0 + [AHL][LuxR]}$$

-

$$d_2[aiiA]$$

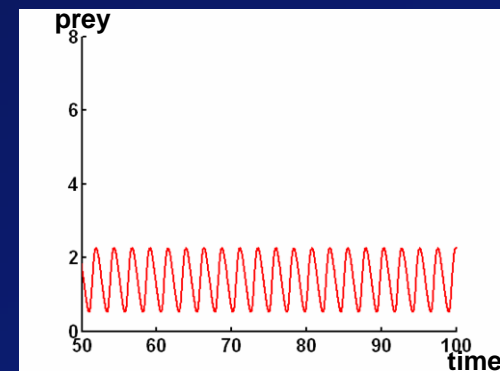
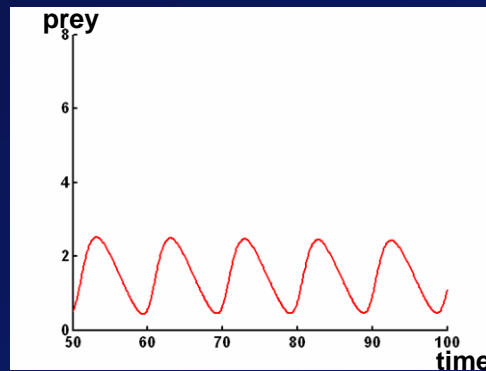
Full System Simulations

**Graph of
Prey vs. Time**

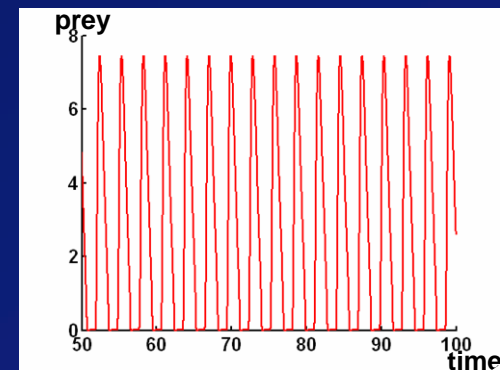
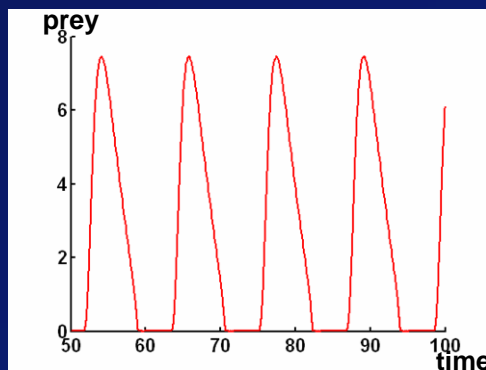
Low Frequency

High Frequency

**Small
Amplitude**

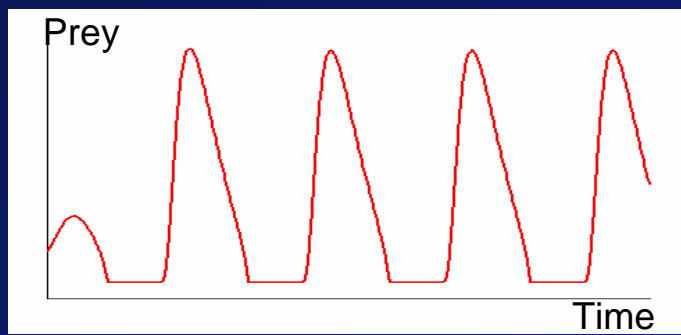
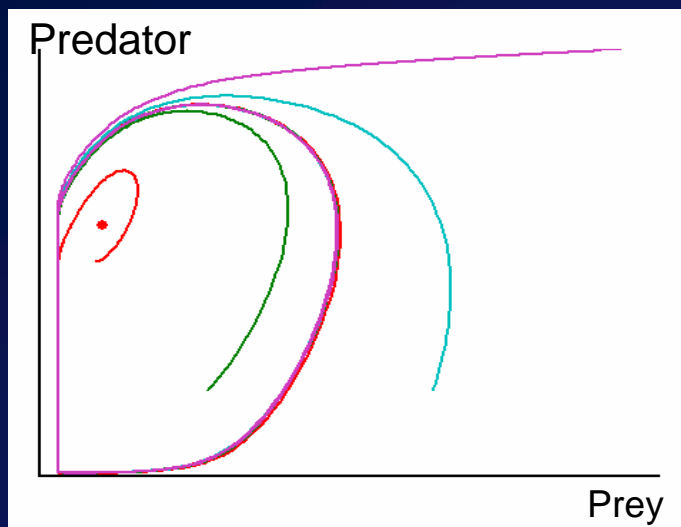


**Large
Amplitude**

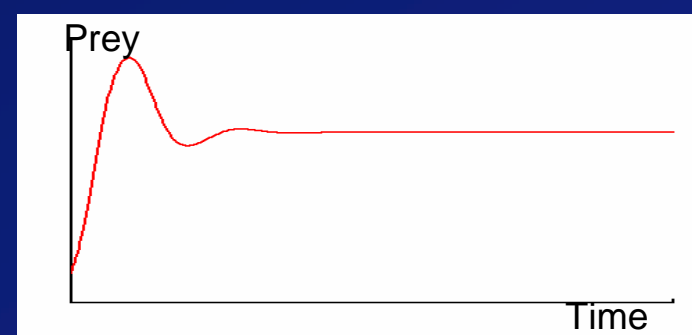
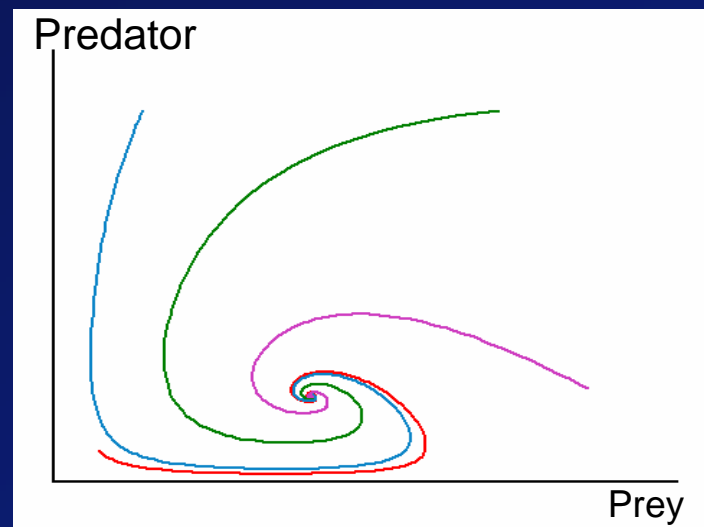


Typical System Behaviours

Oscillations with limit cycles



No oscillations





Modelling the Full System

Population dependent

$$\frac{d[AHL]}{dt} =$$

Gene Expression

$$\frac{a[AHL]}{a_0 + [AHL]}$$

Enzymatic Reaction

$$\frac{b[aiiA][AHL]}{b_0 + [AHL]}$$

Degradation

$$e[AHL]$$

$$\frac{d[LuxR]}{dt} =$$

$$\frac{c[AHL][LuxR]}{c_0 + [AHL][LuxR]}$$

—

$$d_1[LuxR]$$

$$\frac{d[aiiA]}{dt} =$$

$$\frac{c[AHL][LuxR]}{c_0 + [AHL][LuxR]}$$

—

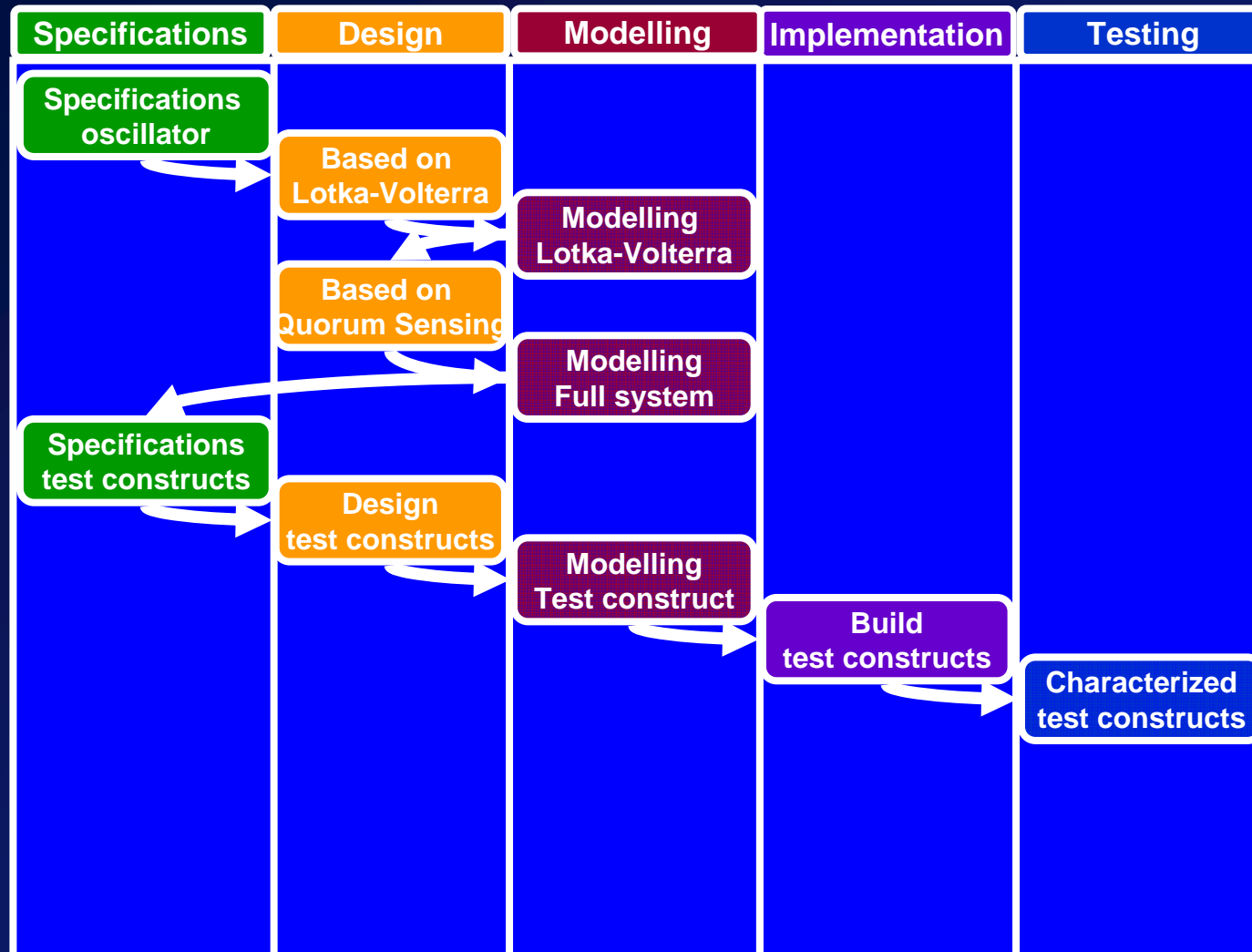
$$d_2[aiiA]$$

Wash-out related

Constant

Path to Our Goal

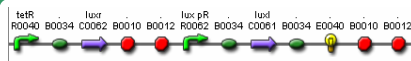
Start!
→



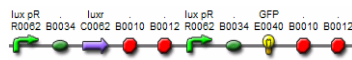


Breaking Down the Complexity

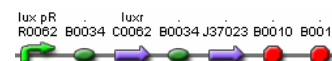
Prey Generator



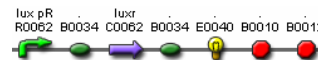
Prey Sensing



Predator Generator



Predator Sensing



Predator Killing





Characterization Predator Sensing

Test part

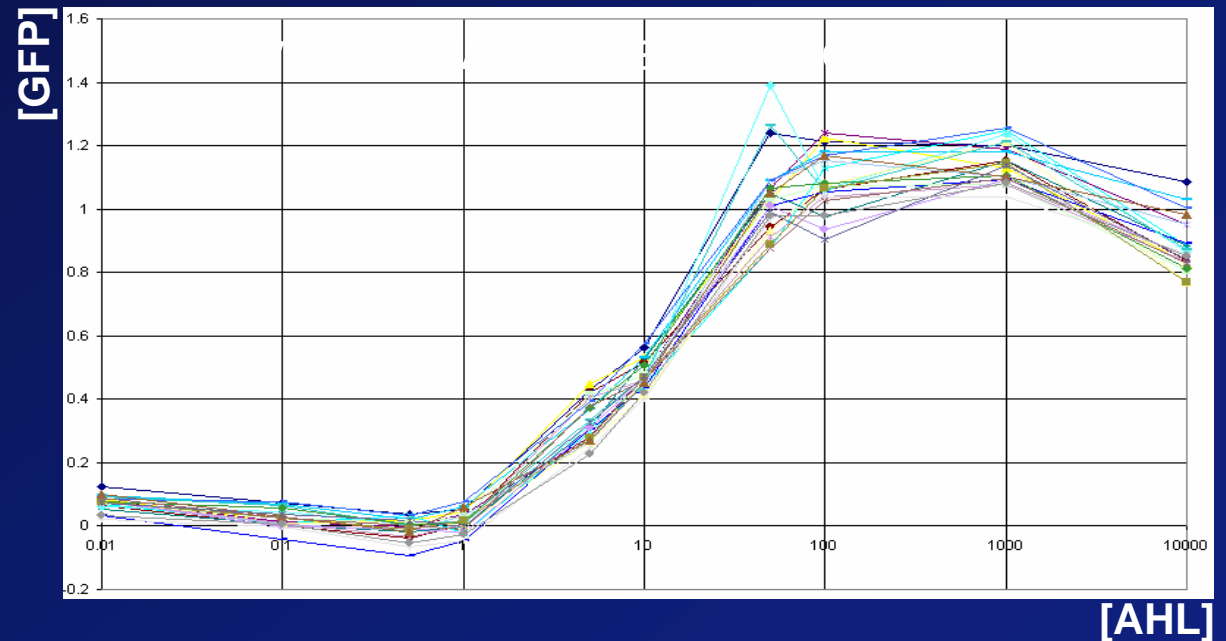


Predictive model
transfer function



Experimental data

Experimental Data





Characterization Predator Sensing

Test part



Predictive model
transfer function



Experimental data

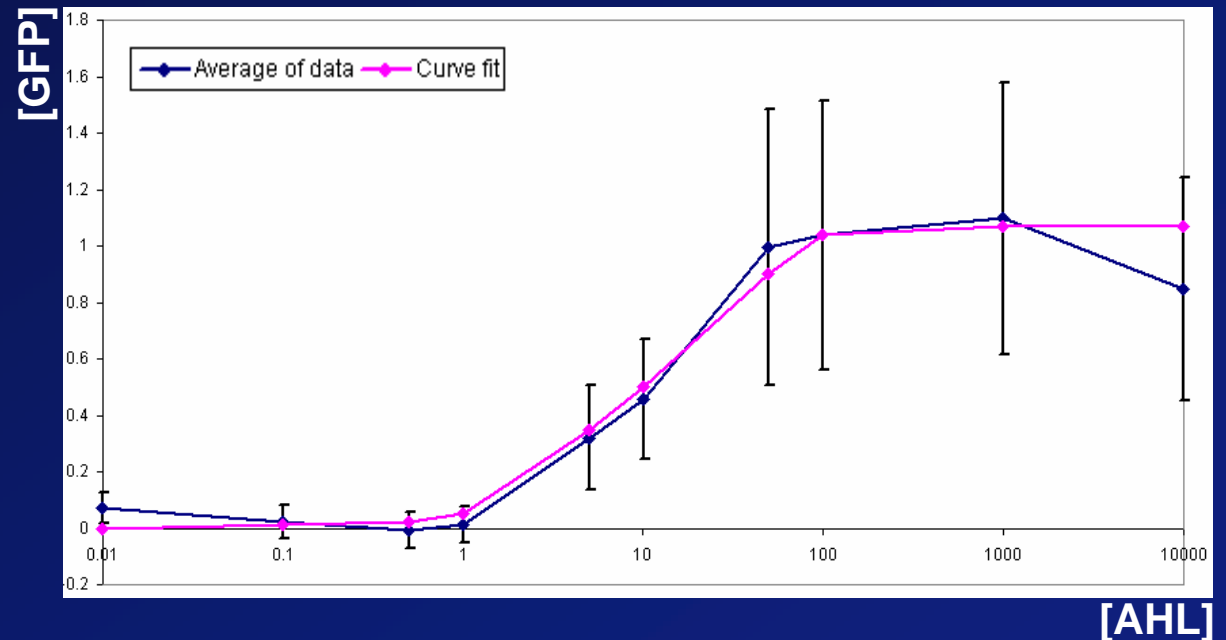


Fitting model to data



Parameter extractions

Average with variance and curve fitting





Implementation

Registry Catalogue Parts

Prey

J37034

RS+J37034

Prey with Riboswitch

J37023

J37024

AiiA Test Construct

J37025

Final predator

J37033

J37019

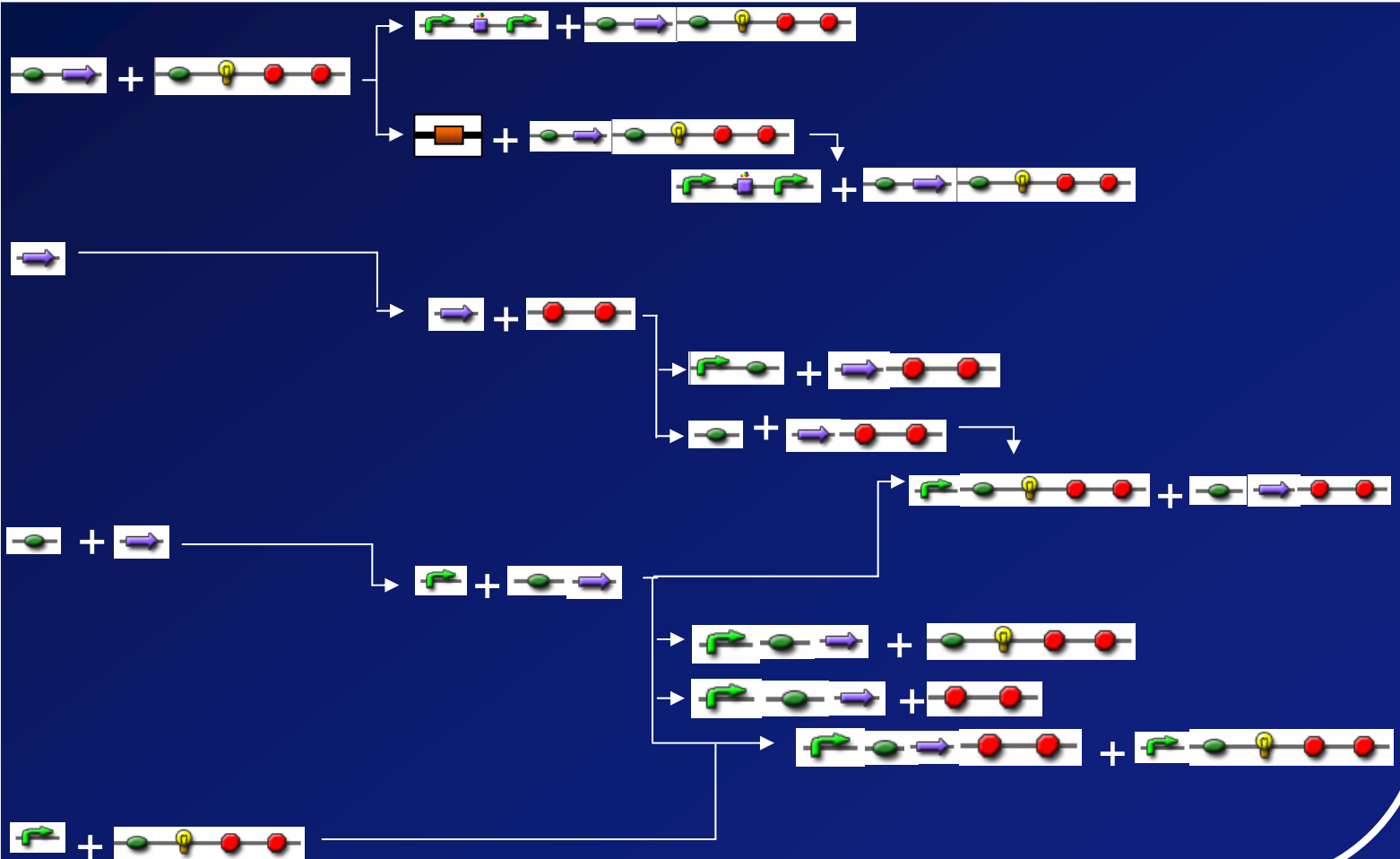
Sensing predator

J37031

Sensing predator

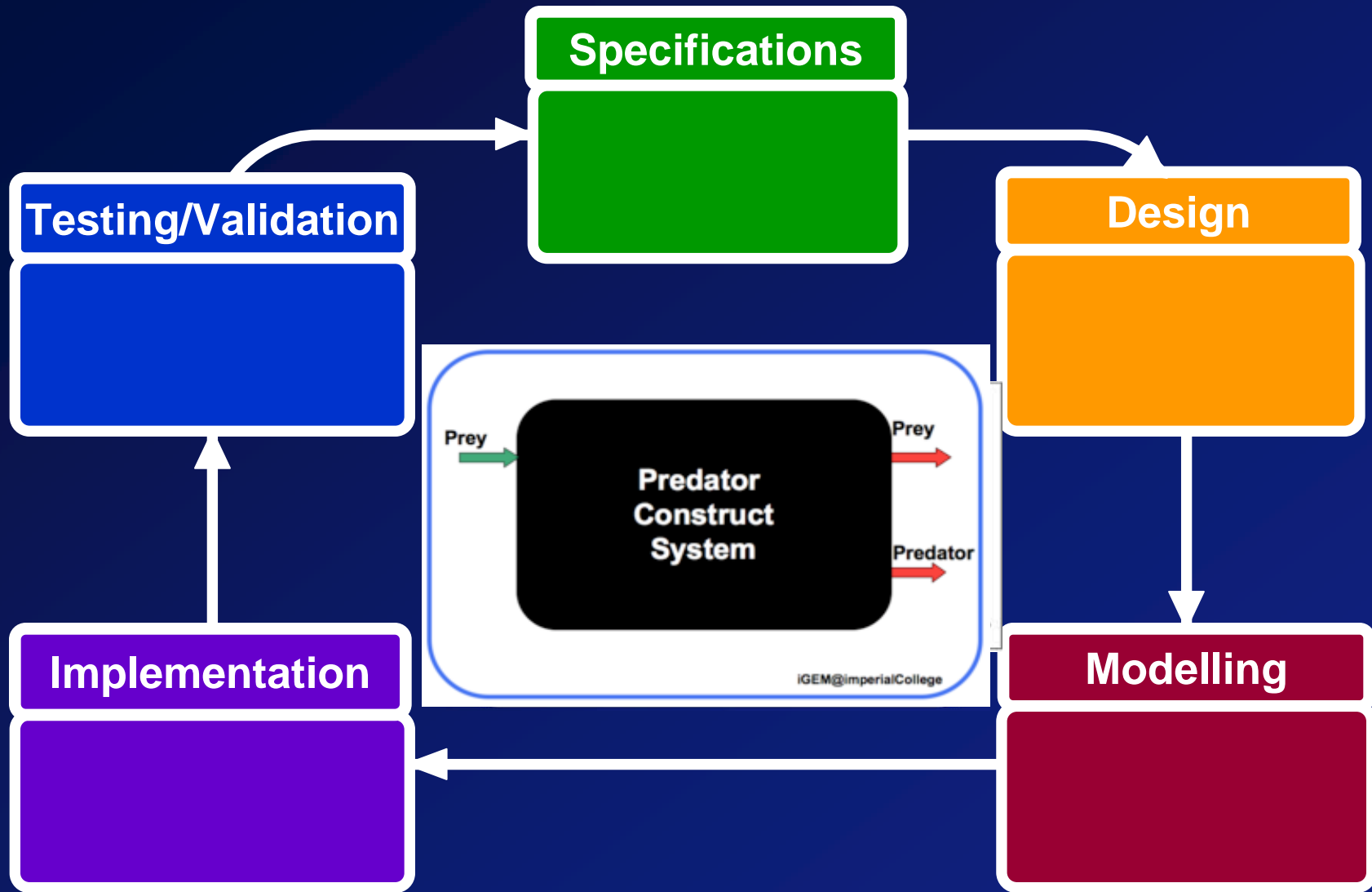
J37032

Assembly process





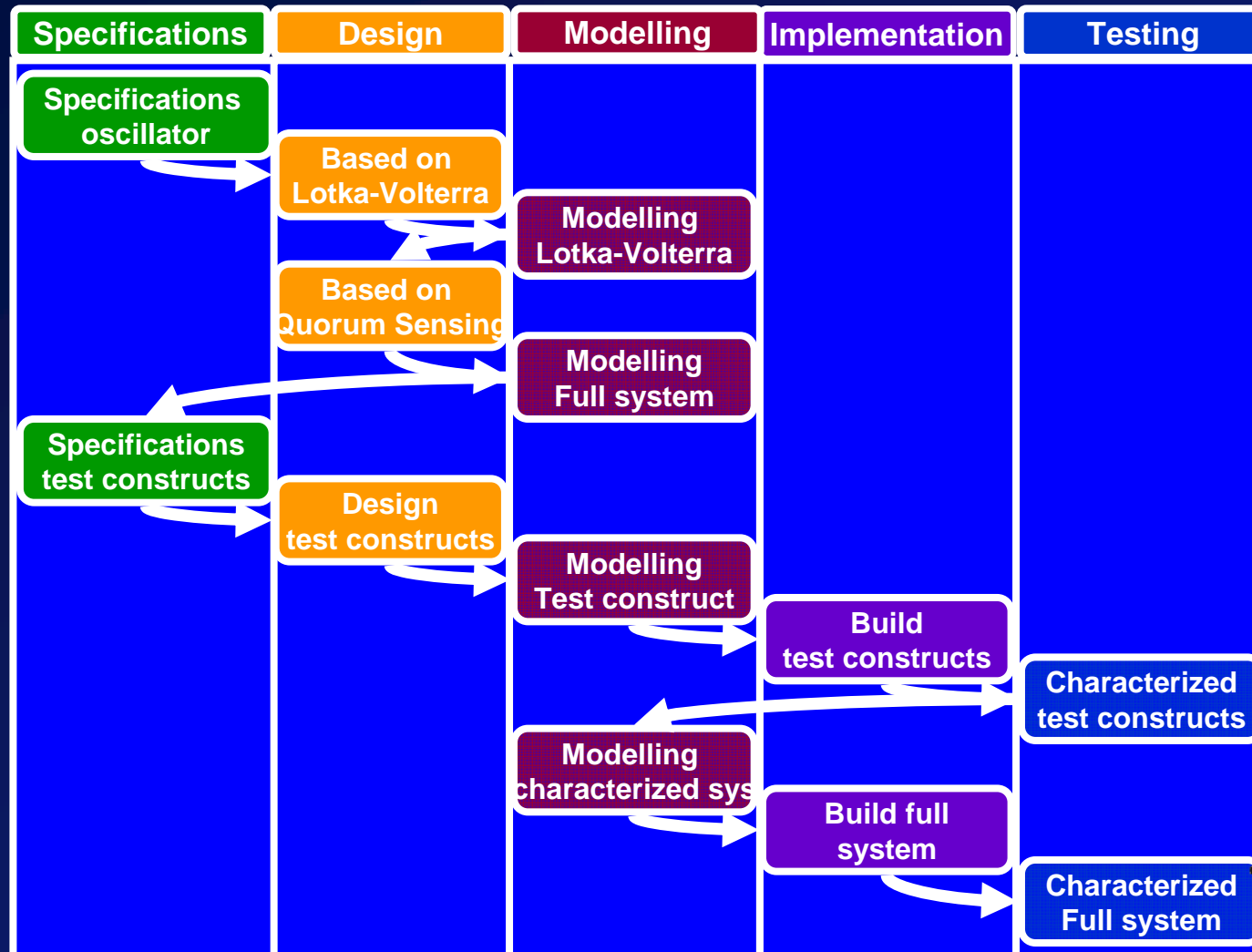
On Our Experience





Path to Our Goal

Start!
→



Our Goal!



Contributions to the Registry

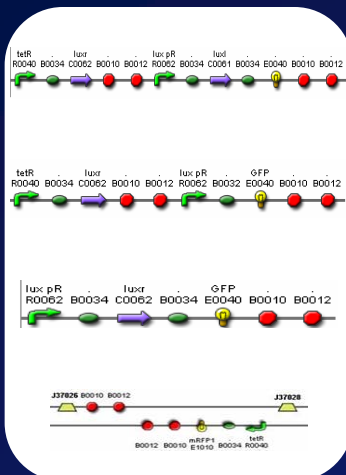
Functional Parts

Final Prey
J37015

Sensing Prey
T9002

Sensing Predator
J37016

Cre/Lox
J37027



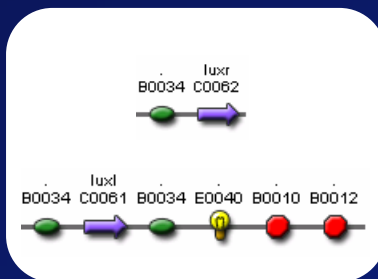
Built Sequenced Tested Characterized Documented

✓	✓	✓	✓	✓
		✓		✓
✓	✓	✓	✓	✓
✓	✓	✓		✓

Intermediate Parts

J37033

J37034



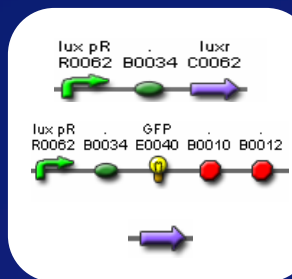
Built Sequenced

✓	✓
✓	✓

J37019

J37032

J37023



Built Sequenced

✓	✓
✓	✓
✓	✓



Our Wiki

Imperial College London
THE iGEM REPORTER
The newsletter of Imperial College London iGEM 2006

[Our Contributions](#)
[To Do List](#)
[Biological Oscillator Parts](#)
[Modelling](#)
[Protocols](#)
[Resources](#)

Breaking News

- 30 Oct 2006: 300+ pages documentation on OWW. New iGEM page
- 27 Oct 2006: Team Poloshirts arrived.
- 26 Oct 2006: BioBricks sent to M.I.T.
- 25 Oct 2006: Presentation of the iGEM 2006 project at the Bioengineering Departmental Seminar.
- News Archive

Location of our visitors
Visits since 6 Aug 2006

Updated daily

Imperial College London iGEM Project(s) 2006

Engineering a Molecular Predation Oscillator.

PoPs Blocker Biological to Electrical Interface

Celebrities

Get to know the I.Coli team and their advisors:

[The Undergrads](#) [show](#)

[The Advisors](#) [show](#)

[Acknowledgements](#)

Activities

- Event Calendar
- MIT Jamboree
- BioSysBio Conference, UK
- UK iGEM Teams Meeting in Cambridge

In the Wetlab

- Lab Notebook
- Protocols
- Lab Status
- Testing
- Sequencing of Parts
- Primers

Education

- Brainstorming
- Journal Club
- Lecture Notes
- Bibliography/Papers
- Resources

Entertainment

- Photos
- Logo & Team Shirts
- Inspirational Quotes
- Project Discussion
- Sandbox

- Documentation
- Communication
- Organization

<http://openwetware.org/wiki/IGEM:IMPERIAL/2006>



Thank You

**Imperial College
London**



Acknowledgements:

- Prof. Tony Cass
- Dr. Anna Radomska
- Dr. Rupert Fray
- Dr. David Leak
- Dr. Mauricio Barahona
- Dr. Danny O'Hare
- Dr. Geoff Baldwin
- Susan E. Wryter
- David Featherbe
- Ciaran Mckeown
- James Mansfield

Funding:

- European Commission
- Imperial College Deputy Rectors Fund
- Faculty of Engineering
- Faculty of Natural Sciences

